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14. ABSTRACT

The Quantum Dot Corporation has recently completed the development of a QD which emits in the near infrared (NIR): QD 705 Streptavidin Conjugate, which can be excited at 633 nm. One step towards using QDs as a functional imaging agent were realized by linking them to immunoglobulin G and Streptavidin where it was demonstrated that the QDs could be used to label the breast cancer marker Her2 on the surface of live cancer cells. This report documents a set of lab experiments on gelatin phantoms that were designed to look at the potential for enhanced imaging of QDs. Our method of choice consisted of using ultrasound in order to "label" them. A bench top system for testing the use of ultrasound to modulate potential specimens was assembled. Recorded data from specimen preparations in the presence and absence of QDs were recorded. Results indicate that the modulated light from QDs can be observed, however is substantially weaker than that observed from the elastically scattered light (at 532 nm). The potential use of Acoustic Modulation for increased resolution of QDs is therefore possible.

15. SUBJECT TERMS
quantum dots, early detection of breast cancer, acousto-optic modulation

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Final Report for Grant W81XWH-04-1-0660
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INTRODUCTION:

This report constitutes the final report for the award given under the Army Breast Cancer Research Program to Dr. Jaffe. The purpose of the award was to fund Dr. Jaffe's work in researching the use of Quantum Dots (Qdots) in order to increase the capability of early breast cancer detection. Under this funding, Dr. Jaffe, and members of his lab, examined the potential use of QDots for fluorescent mapping of the distribution of receptor sites at locations where QDots would bind based on the conjugation of antibodies.

BACKGROUND INFORMATION AND PROJECT RATIONALE:

With the recent invention of the highly fluorescent nanocrystals; quantum dots (QDs), there has been interest in their use in various applications related to breast cancer. QDs have been used in a lab based assay of cell motility for estimating metastatic potential [1], and their use has also been demonstrated as an aid in guiding sentinel lymph node biopsy [2]. The Quantum Dot Corporation has recently completely the development of a QD that emits in the near infrared (NIR): QD 705 Streptavidin Conjugate, which can be excited at 633 nm. One step towards using QDs as a functional imaging agent were realized by linking them to immunoglobulin G and Streptavidin where it was demonstrated that the QDs could be used to label the breast cancer marker Her2 on the surface of live cancer cells [3]. The great advantage of the NIR QD is that both 633 and 705 nm are "reasonable" wavelengths for propagating light through breast tissue [4]. In addition many high quality CCD cameras have excellent sensitivity at both of these wavelengths, which has the potential to make the possibility of breast imaging of NIR QDs at centimeter depths a reality.

We envision a low cost, low risk, biological assay for the presence of early stage breast cancer which takes advantage of the fluorescence properties of QDs in conjunction with some immuno-assay, or the like. One of the great advantages of QDs is that a host of different fluorophores can be excited by the same wavelength. If different QDs are conjugated with moieties that have different biochemical affinity, color images can then be created which show the distribution of sites within different types of cells. The great potential here would be to detect certain types of cell lines. The multiple marker molecules would increase specificity. Our particular interest is in the characterization of an imaging system that could be used to image QDs in the breast. Several groups are working on the biochemical aspects of this problem, however, the imaging properties of QDs which are located in tissues of cm thickness have not been studied in a systematic way (or, at the least, the author of this proposal is not aware of

results that have been published on this topic). Our optimism in achieving this goal is based on the results of [5] who demonstrated the use of CCD cameras for imaging NIR wavelengths in a diffuse optical tomographic (DOT) geometry. Furthermore, [2] demonstrated that only 5 mW of NIR laser stimulation was capable of imaging 400 pmols of QDs at depths of 1 cm with modest CCD camera technology.

Another promising technique for breast imaging has been the recent development of Acousto-Optic (AO) methods for localizing optical scatters [6]. The basic idea of this new and promising method is to combine the diagnostic capability of optical imaging with the localization properties of ultrasound. For the most part, diffuse optical imaging of breast has been limited to a resolution of ~ 1 cm. Unfortunately, this resolution is too low for guided biopsy where suspect areas can be as small as 1 mm. Fortunately, however, ultrasonic resolution in breast is routinely available at millimeter resolution, however, diagnostic ultrasound is incapable of differentiating tumor from normal tissue. This is because ultrasonic backscatter has only marginal dependence on the properties of the suspect tumor and has little reliability for distinguishing between either malignant or pre-malignant tissue and normal. AO methods hold promise for providing diagnosticians with the specificity of optical imaging, say for measuring blood oxygen level, with the resolution of ultrasound (mm). Recent work in this area has been successful in obtaining increased detection capability [7, 8] with the use of photorefractive crystals. Our optimism was, in part, guided by the recent results of Swan et al [9] who demonstrated that it was possible to obtain superior resolution in fluorescence imaging via the self-interference of quantum dots. Although previous work [6] seemed to indicate that coherent illumination was necessary for detection, the self coherence of a single dot might lead to an observable effect.

Based on the potential use of AO modulated imaging of diffuse scatter, we undertook to perform a number of lab experiments whose goal was the examination of the potential use of QDs in combination with AO modulation in order to obtain increased resolution imaging for localizing QDs in vivo. The method consisted of embedding QDs in a gelatin phantom and then using a synchronized source and receiver in order to see if QD imaging was possible. Given the available hardware in our lab we decided to implement the parallel detection scheme of Leveque et al. [10] that requires the synchronization of a light source with the incident ultrasound beam. In this way, a CCD camera can be used in order to implement a parallel detection scheme that allows a great degree of averaging to occur in viewing these small amplitude and low Signal-to-Noise (SNR) signals.

TECHNICAL WORK ACCOMPLISHED:

In order to perform the lab experiments we assembled a “test bed” system for observing the resultant AO modulation of tissue in both the presence and absence of QDs. Figure 1 shows a diagram of the lab setup for the electro-optical system, including laser source, AO modulator, sample position, ultrasonic source, and the detection camera. As shown, after passing the laser beam through an AO modulator (Model: 1080 – 125, Crystal Technologies, Palo Alto, CA) the diffracted light is incident upon a gelatinous sample. The sample was prepared by dissolving ½ teaspoon of Gelatin (Knox Original Gelatine, Kraft Foods, Tarrytown, NY) into approximately 10 ml of heated distilled water. To this, several drops of 1-micron polystyrene spheres were

added (exact concentration unknown). The solution was stirred and allowed to set in a refrigerator overnight.

QDs were purchased from Molecular Probes Inc. (29851 Willow Creek Road, Eugene Oregon). We used the Qtracker 655 non-targeted quantum dots in a 2 micro-M solution. Such dots could be stimulated with our existing 30 mW 532 laser (Kentek DMG532-30, Pittsfield, NH) and observed with a red filter on a CCD camera at a wavelength of 653 +/- 14 nm. As such, we used a filter at 645 nm, well within the emission band of these QDs.

In order to view the QDs, 40 microliters of the Qtracker 655 non-targeted quantum dots solution was injected into a small area. Upon illumination with the green light, the q-dot was clearly visible as a bright red disk due the fluorescence emission at 655.

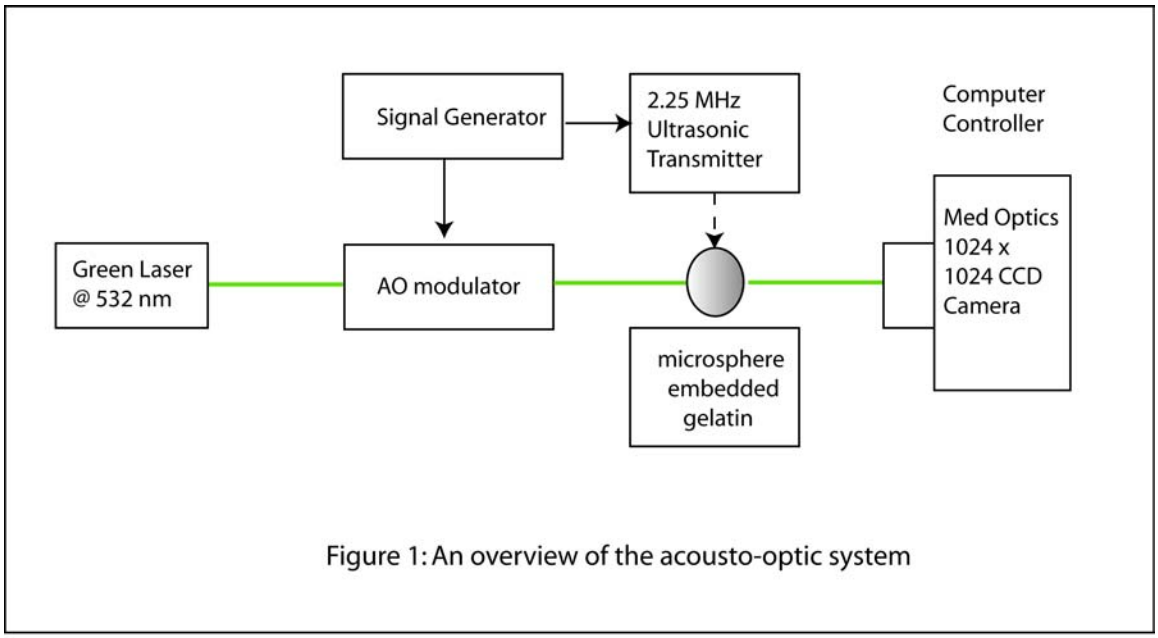


Figure 1: An overview of the acousto-optic system

The collected data consisted of several hundred CCD images that were synchronized with the acoustic signal via the signal generator (Figure 1). The AO modulator was also synchronized with the acoustic signal in order to subdivide the collected CCD images into four sets that were "triggered" at different phases of the acoustic signal. The data integrated from each set of images was then processed in order to estimate both the phase and the intensity of the resultant speckle pattern [10].

The basic model of AO modulation of tissue starts with an assumption that the complex field of the light incident on the CCD chip can be understood as consisting of both an unscattered or DC component and a scattered, or AC component.

$$E = E_0 + E_1 \exp(i\omega t + \varphi)$$

where ω is the angular frequency of the ultrasonic field and φ is an arbitrary phase due to randomness in path length and changes in refractive index, and hence the transit time of the

pulse. The intensity of the received light can be viewed (after making several approximations) as

$$I = I_0 + I_1 \exp(i\omega t + \varphi)$$

where I is the intensity measured on the CCD camera and the variables are as described above. In order to observe the AO effect, we estimated both the I_0 (the unmodulated component) and I_1 (the modulated component) with the ultrasonic beam turned off and then turned on. A systematic increase in I_1 over I_0 indicated the presence of the AO effect, whereas an absence of increase of I_1 over I_0 indicated that the AO effect was either negligible or unobservable for some other reason. As shown in [10] an estimate of I_1 can be computed via a simple analysis of the four sets of images that were taken with different phase offsets between the acoustic beam and the incident pulsed (AO modulated) energy.

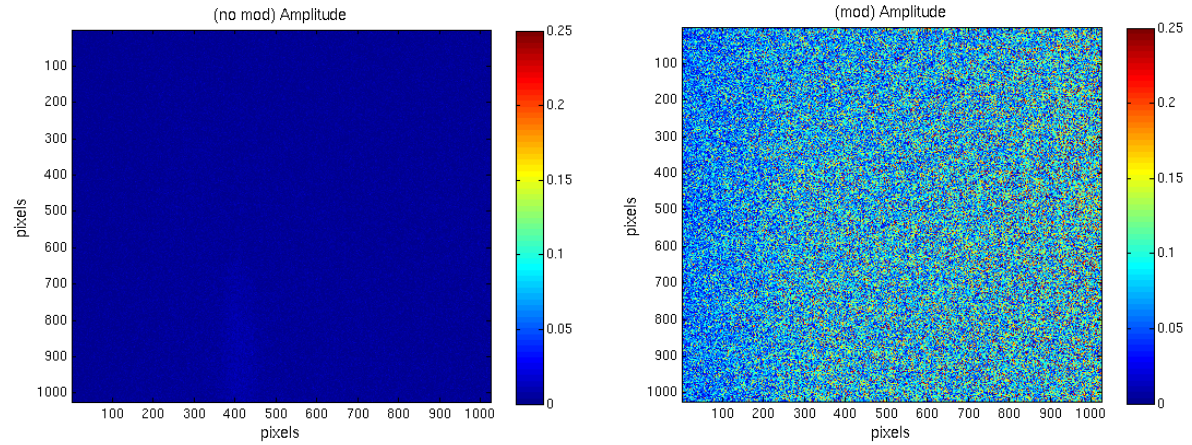
In order to control the experimental collection system custom LabView code (National Instruments, Austin TX) was written in order to aid in the data collection process. Because of observed temporal decorrelation in the sample (time varying behavior) a set of four images was taken in sequence, one for each phase. The amplitudes of the modulated and nonmodulated components were then estimated for each of these four images and the resultant set of nonmodulated versus modulated images were then displayed.

EXPERIMENTAL RESULTS

A single experiment consisted of putting either a QD or non-QD specimen into the place where both optical and acoustic energy was incident. The filter used to control the wavelength of light incident on the CCD camera was also changed in order to limit the collected light to either the green light for the simply scattered and modulated photons or the red light for the QD scattered energy. Subsequently the resultant optical pattern onto the CCD camera was recorded and then analyzed in order to estimate the intensity of the unmodulated and modulated beam components in addition to the phase for each element of the CCD camera. Results are displayed as a set of images which display the estimate of the intensity versus position on the CCD camera.

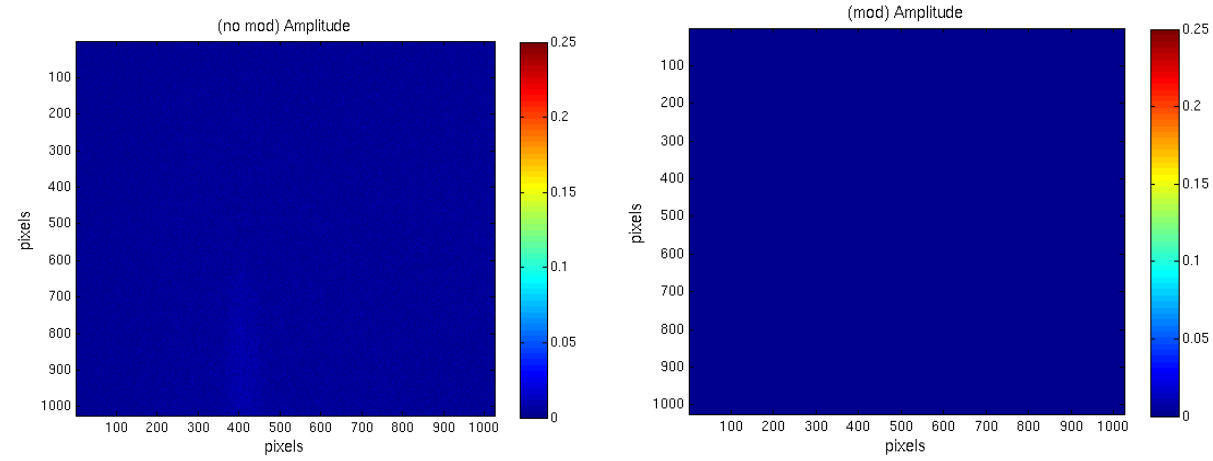
Resultant Images for the QD and NonQD light

The basic results indicate that, in our experimental setup, a strong modulation effect can be observed for the elastically scattered photons (532 nm). The observed effect for the QDs was observable, however small. Figure 2 a and b shows the estimated amplitudes of the modulated component of the light in the absence (a) and presence (b) of acoustics. The obvious effect is that the acoustically modulated energy is readily discernable in this comparison. Figure 3 shows the two sets of images that were obtained when looking at the red light that exited from the specimen at 645, due to the presence of the QDs. It is clear that the effect here in only marginal.



(a) (b)

Figure 2: The resultant estimated amplitude plots for the elastically scattered light (532 nm) in the (a) presence and (b) absence of modulation. The resultant effect of the modulation at this wavelength is extremely large and measurable.



(a) (b)

Figure 3: The resultant estimated amplitude plots for the QD fluoresced light in the (a) presence and (b) absence of modulation. The resultant effect of the modulation at this wavelength is much weaker than observed for the light at 532 nm.

Data was also analyzed by taking the ratio of the non modulated to the modulated amplitudes for both the QD and non QD case. The results are shown in Table 1, which compares the four cases.

	Unmodulated	Modulated
No Quantum Dots	236.3	11.34
Quantum Dots Present	974.5	971.5

Table 1: A comparison of the ratio of $\frac{I_0}{I_1}$ *(unmodulated to modulated photons) in the presence or absence of quantum dots. The results indicate a "weak" effect for the quantum dots and a "strong" effect for the inelastically scattered photons*.

Signal and Image Processing Techniques

In concert with observations of other researchers, we observed the AO modulation effect to be very small. That is to say, the ratio of the AO unmodulated energy relative to that of the modulated energy was large. For example, as above, in the case of the elastically scattered photons, the ratio of the energy in the modulated to unmodulated was still 11.32, indicating that less than 10% of the photons were modulated. This makes the signal extremely difficult to see and has motivated the use of more exotic techniques such as photorefractive crystals [7,8].

In order to combat this low SNR we decided to apply some standard image processing techniques to the observed CCD images that used some a-priori knowledge related to both the spatial and temporal characteristics of the images. These consisted of using the redundancy in the four images and the implementation of spatial filters for detection. Both techniques resulted in an increase in SNR (10% – 20%).

In the case of a-priori knowledge of the signal, we used the fact that we were measuring four images, however were estimating only three quantities (two intensities and one phase). As is well known, over determining an inverse problem typically results in an increase in the accuracy of the estimated data. This was certainly true in our case. This led to a more stable estimate of the relative intensities of the modulated and unmodulated components in addition to the phase.

In the case of the image processing techniques, we initially observed that the noise inherent in our signal and thus in our estimation technique was dominated by the counting statistics, or "shot noise" inherent in our data. Predictions of the effects of standard shot noise, that is, signal dependent noise indicated a strong noise component that displayed this effect. We therefore employed some standard techniques to increase SNR in the image estimates of the nonmodulated to modulated components. Both median filter and also logarithmic processing algorithms were tried with some increase in SNR, as measured by the inherent variability of the data. We concluded in this phase of our work that using space-time processing methods, that the resultant estimates could, in fact, be enhanced.

KEY RESEARCH ACCOMPLISHMENTS:

- Suggested Use of Quantum Dots to Enhance resolution of Breast Imaging when combined with Acoustic Modulation Techniques.

- Demonstrated Modulation (weak) of Quantum Dot light output using Acoustic Modulation.
- Development of Signal and Image Processing Methods to Increase SNR in AO modulated imaging of Quantum Dots.
- Development of Experimental Methods to Accommodate Temporal Non-stationarity in AO modulation of tissue.

REPORTABLE OUTCOMES:

Increased collaborative arrangements between Scripps Institution of Oceanography and the UCSD Medical School: As a result of the funding available to Dr. Jaffe under this grant, he has been actively working with members of the UCSD medical school. This past spring they authored a major proposal to NIH. As with any proposal, a large amount of work was put into thinking about the potential for new work. The particular focus of this proposal was to create a next generation diagnostic machine that could combine both acoustics and optics in order to achieve higher accuracy in early detection of breast cancer. The goals of this proposal were therefore aligned perfectly with the research sponsored under this program. Based on results obtained under this program and our experience in both the optical use of QDs and their potential interaction with sound, we plan to pursue future funding options for this research as well.

Exposure of Scripps Institution of Oceanography staff and students to biomedical engineering: As part of this program we have naturally used our lab resources in order to accomplish the results outlined above. This has included the exposure of several of our staff and students to this field of biomedical diagnosis and engineering. This provides broader exposure to these personnel in their consideration of future careers. Also, the cross fertilization of our lab methods with those in biology have resulted in a positive outcome for both fields.

CONCLUSIONS:

In this research we have considered an experimental method whose aim is to increase the resolution available to diagnosticians interested in the early detection of breast cancer. The method that we chose to explore was the use of acoustic modulation of light in order to increase the spatial resolution of diffuse optical methods from ~ 1cm to 1 mm. This resolution increase has important clinical implications because many tumors can start out by being quite small. Our results are promising, however, somewhat inconclusive in that we observed a small effect of the acoustic modulation. Additional benefits of the research can be derived from the techniques that we have developed for processing acoustically modulated images such as space-time filtering.

If additional work demonstrates that our preliminary findings can be statistically validated, then, a future generation of diagnostic techniques for breast cancer early detection and diagnosis will be greatly aided. Potential benefits range from both acousto-optically guided needle biopsy to an increased ability to spatially locate and characterize breast cancer. This early detection and localization would present a great benefit.

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